

Utility Spectrophotometric and Chromatographic Methods for Determination of Antidepressant Drug Sulpiride in Pharmaceutical Formulations and Plasma

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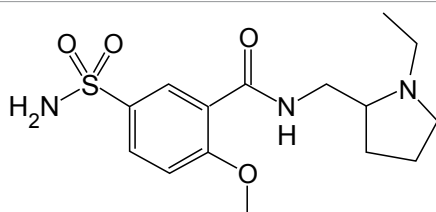
Abstract

Validated spectrophotometric and chromatographic methods have been developed for determination of the antidepressant drug sulpiride (SUL) in pharmaceutical formulation and plasma. The new spectrophotometric methods were based on the formation of sulpiride yellow ion-pair complex with bromocresol green (BCG), congo red (CR) or methyl orange (MO) in Britton-Robinson universal buffer of pH 3.0, 5.0 or 2.5, respectively. The formed complexes with BCG, CR and MO were extracted with chloroform and their absorbencies were measured at 420 nm, 515 nm, and 480 nm, respectively. Beer's law was obeyed over the concentration ranges of 2-14.0, 2-16.0, and 2-14.0 µg/mL sulpiride with BCG, CR and MO, respectively. The molar absorptivity (ϵ) of the formed colored complexes with BCG, CR and MO was 4.10×10^4 , 2.10×10^4 and 3.50×10^4 L mol⁻¹ cm⁻¹ and the estimated limit of detection (LOD) of sulpiride was found to be 0.044, 0.095 and 0.064 µg/mL, respectively. In the developed high performance liquid chromatographic method (HPLC), quantitation of sulpiride was carried out on C₁₈ reversed phase column (250×4.0 mm, 5 µm) using a mobile phase of acetonitrile: methanol: water: Britton-Robinson (B-R) universal buffer of pH 9 (20: 20: 40: 20, v/v/v/v) delivered at a flow rate of 0.6 mL/min with UV-detection at 225 nm. Calibration graph of bulk sulpiride was linear over the concentration range of 0.034-110 µg/mL. The described spectrophotometric and HPLC methods have been applied successfully for the analysis of sulpiride in its dosage form without interference from common excipients. Statistical comparison of their results with those obtained using a reported membrane selective electrode method showed excellent agreement and indicated no significant differences in accuracy and precision.

Keywords: Sulpiride; Dogmatil fort®; Spectrophotometry; Ion pair complexes; HPLC determination

Introduction

Antidepressant drugs are widely used for treatment of depression and they are frequently encountered in emergency toxicology screening, drug-abuse testing and forensic medical examinations [1]. Sulpiride; SUL, Scheme 1, (5-(aminosulfonyl)-N-[(1-ethylpyrrolidin-2-yl)methyl]-2-methoxybenzamide) falls into the large group of antidepressant drugs [2,3]. It possesses anti-psychotic, antidepressive, and antiulcer effects. It has peculiar affinity for the D₂ and D₄ brain dopamine receptors with a low frequency of extrapyramidal side-effects [4]. SUL also exhibits neuroleptic and thymoleptic properties and is used in mental disorders as a behavior regulator in the psychopathology of senescence, in depression, and in schizophrenia. It is also used in the treatment of gastric or duodenal ulcers, in the treatment of irritable colon due to psychosomatic stress, and in various vertigo syndromes.



Scheme 1: Chemical structure of sulpiride.

Several methods have been developed for detection of SUL, including spectrofluorometric [5,6], spectrophotometric [7-10], gas chromatography [11], liquid chromatography [2], thin layer chromatography [7-12], high performance liquid chromatography (HPLC) with fluorescence [13-17], or mass spectrometric detection [3],

radioimmunoassay [18,19], voltammetric [20,21], membrane selective electrode [22], capillary electrophoresis with electrochemiluminescence (CE-ECL) and ultraviolet [23-25] and H-1-NMR spectroscopy [26].

Most of these methods involved determination of SUL in two component mixture [7,9,10,18,24,26] or in human biological fluids [2,3,5,6,11-17]. A non-aqueous titration method for determination of SUL in bulk and in pharmaceutical tablets was described in British [27] and European [28], Pharmacopoeias.

Ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds [29-33], for its sensitivity and capability for offering distinct possibilities in the assay of a particular component in a complex dosage formulation. On the other side, most reported high performance liquid chromatography [3,13-17] necessitate sophisticated detection, lengthy extraction steps with organic solvents prior to assay of analyte, long run time (\approx 25 min), sensitive ion-pair reagents [34,35]. All these reported

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HPLC are time-consuming and not economically feasible for routine use in pharmacokinetic studies with numerous samples to be analyzed.

This paper aimed to describe a spectrophotometric method for determination of SUL in pharmaceutical formulations and plasma based on extraction of its soluble ion-pair complexes with some acid dyes in buffered solutions. Besides, a rapid HPLC-UV method without ion-pair reagent in mobile phase was also described.

Materials and Methods

Material

Bulk sulpiride (SUL) was obtained from Memphis CO. for Pharm. & Chem. Ind., Cairo, Egypt. Dogmatil fort® tablets claimed to contain 200 mg SUL per tablet (Memphis CO. for Pharm. & Chem. Ind., Cairo, Egypt) were purchased from the local market. Sodium salt of each of the acid dyes bromocresol green (3,3',5,5'-Tetrabromo-*m*-cresolsulfonphthalein), congo red (Benzidinediazo-bis-1-naphthylamine-4-sulfonic acid) and methyl orange (p-Dimethylamino-azobenzenesulfonic acid) were obtained from Merck (Darmstadt, Germany).

Solutions

(a) A stock solution (200 µg/mL) of bulk SUL was prepared by dissolving 20 mg of pure drug in de-ionized water, transferring it into a 100 ml measuring flask, and diluting it with water up to the marking. Working standards (0.01 to 130 µg/mL) were prepared by serial dilutions with the mobile phase: acetonitril: methanol: water: B-R universal buffer of pH 9 (20: 20: 40: 20, v/v/v/v).

(b) Solutions of 1×10^{-3} mol/L of each of bromocresol green (BCG) congo red (CR) and methyl orange (MO) were prepared by dissolving accurate weight of the acid dye-sodium salt in a few drops of methanol and then in de-ionized water in 100 mL-volumetric flasks.

(c) A series of the Britton-Robinson (B-R) universal buffer of pH 2-11 were prepared [36], in de-ionized water. All the chemicals used were of analytical-reagent grade quality and were used without further purification. The pH of solutions was checked using an Orion Research digital pH-meter Model 601A (Yokohama, Japan). De-ionized water was obtained by a Purite-Still Plus de-ionizer connected to an AquaMatic double-distillation water system (Hamilton Laboratory Glass LTD, Kent, UK).

Spectrophotometric measurements

Apparatus: Adsorption spectral measurements were made using UV-Visible spectrophotometer (Jasco, Hachioji-cho, Tokyo, Japan) with 10 mm quartz cells.

General procedure: Aliquots of the standard solution of SUL were transferred into a series of reaction flasks followed by the addition of 1.5 mL, 2.6 mL, and 2.0 mL (1.0×10^{-3} mol/L) of BCG, CR or MO, respectively, the total volume of the aqueous phase was adjusted to 10 ml by the B-R universal buffer solution of selected pH (Table 1), then mixed well (the final concentration of SUL was in the range of 0.1 to 100 µg/mL). After 2 min vortexing the flasks were allowed to separate the two layers by centrifugation. Each of the formed yellow ion pair complexes was extracted with 10 mL chloroform. The chloroform layer was dried by running through anhydrous sodium sulfate. Absorbance of each of the yellow-colored ion pair complex was measured at λ_{\max} shown in Table 1 (after standing for 5.0 min in each case) against a reagent blank similarly prepared. A calibration graph of absorbance versus concentration of the SUL was plotted.

Parameter	Values		
	BCG	CR	MO
pH	3.0	5.0	2.5
Extracting solvent	chloroform	Chloroform	chloroform
λ_{\max}	420	515	480
Molar ratio (SUL : Dye)	1 : 1	1 : 1	1 : 1
Beer's law limits (µg/mL)	2-14	2-16	2-14
Molar absorptivity (L mol ⁻¹ cm ⁻¹)	4.1×10^4	2.1×10^4	3.5×10^4
Sandell's sensitivity (ng cm ⁻²)	10.2	26.3	12.3
Range of error %	0.74 : -0.60	0.51 : 0.24	0.86 : -0.91
Regression equation*			
Intercept	0.236 ± 0.0018	0.184 ± 0.0022	0.016 ± 0.002
Slope	0.098 ± 0.003	0.038 ± 0.002	0.081 ± 0.003
Correlation coefficient (r)	0.9990	0.9991	0.9998
Limit of quantification (µg/mL)	0.15	0.30	0.20
Limit of detection (µg/mL)	0.044	0.095	0.064
PKf	5.50	4.25	4.80

*A=a+bC, where C is the concentration in µg/mL

Table 1: Quantitative parameters for spectrophotometric determination of SUL as ion-pair complexes with BCG, CR, and MO.

Chromatographic measurements

Apparatus: A liquid chromatographic pump (Bischoff, Switzerland) equipped with a UV-detector (Bischoff Lambda 1000) and a reversed phase column (Prontosil C₁₈, 250×4.0 mm, 5 µm) were used. Data acquisition and peak integration was done with the Bischoff McDAcq integrator software v1.5. The injection volume was 20 µL with a Rheodyne 7125 injector valve.

Absorption spectra of SUL determination in pharmaceutical formulations and plasma was recorded at room temperature within the wavelength range 200-600 nm using a Shimadzu UV-visible spectrophotometer Model 160A (Kyto, Japan). From the UV spectra of the analyte, the detection wavelength was chosen as 225 nm.

HPLC procedure: Sulpiride was quantitated on a C₁₈ reversed phase column, however the mobile composition was, acetonitril: methanol: water: B-R universal buffer of pH 9 (20: 20: 40: 20, v/v/v/v) delivered at a flow rate of 0.6 mL/min at ambient temperature of $25 \pm 2^\circ\text{C}$, and with UV detection (wavelength=225 nm). The mobile phase was sonicated-well before use and the column was equilibrated with the mobile phase flowing through the system before the injection of the standard solution of the analyte. Each standard solution was injected in the chromatographic system ($n=3$) and mean values of peak areas (A) were plotted against concentrations (C).

Assay procedure for tablets: Ten tablets of dogmatil fort® were weighed and the average mass per tablet was determined, and then ground to fine powder. A weighed portion of the homogeneous powder equivalent to 200 µg/mL SUL was accurately transferred into a 100 mL volume calibrated flask containing 70 mL water. The content of the flask was sonicated for about 10 min and then filled up with water. The solutions were then filtered through a 0.45 µm Milli-pore filter (Gelman, Germany). Convenient concentrations of SUL were then obtained by accurate dilutions with de-ionized water (for spectrophotometric measurements) or with the mobile phase: acetonitril: methanol: water: B-R universal buffer of pH 9 (20: 20: 40: 20, v/v/v/v) (for HPLC measurements). Thereafter, the general procedure was followed.

In vitro assay of sulpiride in plasma: To 5 mL plasma contained in three separatory funnels add different volumes of SUL standard

solution prepared in distilled water (0.2 mg/mL). Extract with two 5 mL portions of chloroform. After separation, collect the chloroformic extracts into a graduated measuring cylinder and evaporate in a water bath until the volume is reduced to 2 mL. Calculate the concentration of recovered drug from a calibration graph.

Results and Discussion

Spectrophotometric studies

Sulpiride (SUL) was found to interact with each of the acid dyes bromocresol green (BCG), congo red (CR) and methyl orange (MO) in acidic media forming yellow ion-pair complexes. These complexes were easily extracted quantitatively into chloroform. The absorption spectra of the extracted ion-pair complexes were recorded within the wavelength range of 300-600 nm against a blank solution (Figure 1).

The formed ion-pair complexes show a maximum absorbance at λ_{\max} depends on type of the acid dye as indicated in Table 1. Hence, this wavelength was used for all subsequent measurements. The optimum conditions for these interactions were established by a number of preliminary experiments as described in the following:

Effect of pH: Influence of pH on formation of the ion-pair complexes of SUL with the examined acid dyes has been studied in B-R universal buffers of different pH values. Maximum development of

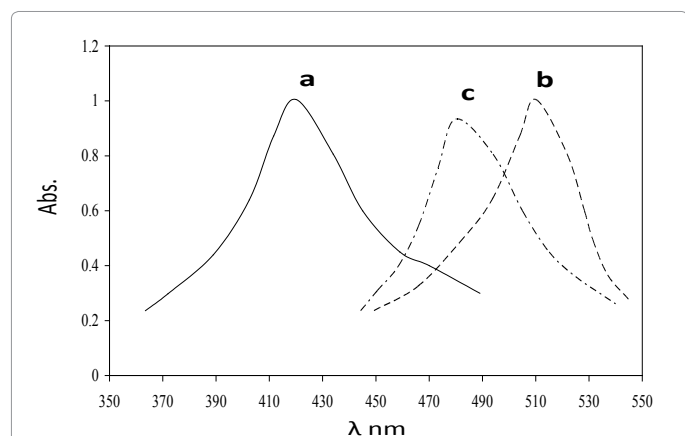


Figure 1: Absorption spectra of: a) SUL-BCG, b) SUL-CR, and c) SUL-MO ion-pair complexes formed with 10 $\mu\text{g/mL}$ SUL.

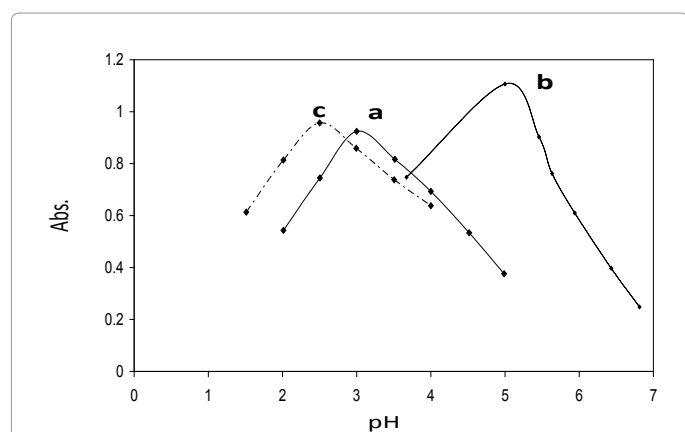


Figure 2: Effect of pH on the absorbance of ion-pair complexes formed with 10 $\mu\text{g/mL}$ SUL: a) SUL-BCG, b) SUL-CR, and c) SUL-MO ion-pairs complex.

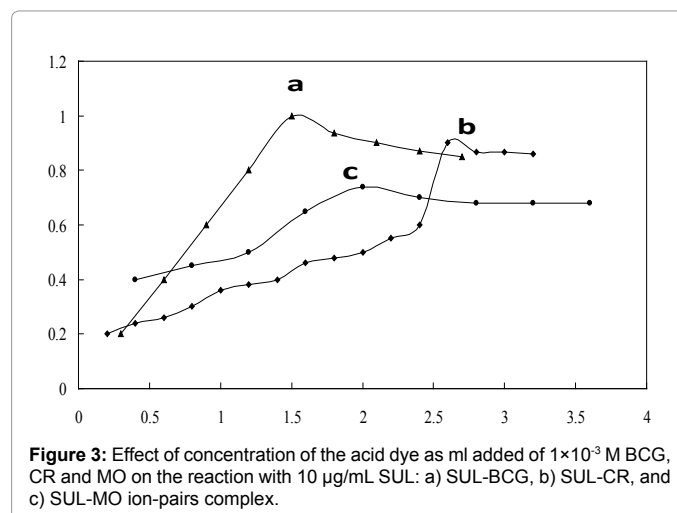
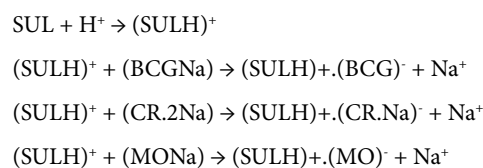


Figure 3: Effect of concentration of the acid dye as mL added of 1×10^{-3} M BCG, CR and MO on the reaction with 10 $\mu\text{g/mL}$ SUL: a) SUL-BCG, b) SUL-CR, and c) SUL-MO ion-pairs complex.

color intensities of the formed SUL ion-pair complexes with BCG, CR, and MO were achieved at the pH values 3.0, 5.0, and 2.5, respectively (Figure 2). Formation of the ion-pair complexes may be attributed to the protonation of the tertiary amino group of SUL in acid medium leading to form ion-pair complex with the anionic dye species. The formed ion-pair complexes were found to extract easily and quantitatively into chloroform. The formation reactions of the ion-pair complexes in the acidic medium can be illustrated in the following:



Effect of dye concentration: Effect of changing of concentration of the examined acid dyes (as mL added) on the development of the color intensity at λ_{\max} of their formed ion-pair complexes with 10 $\mu\text{g/mL}$ of SUL was examined (Figure 3). The results indicated that the maximum absorbance of the formed ion-pair complexes of SUL was achieved on the addition of 1.5, 2.6, and 2.0 mL of 1×10^{-3} M of each of BCG, CR, and MO reagents, respectively, which were used for formation of the ion-pair complexes of SUL throughout the rest of this analytical work.

Choice of organic solvent: Quantitative extraction of the formed SUL-Dye ion pair complexes from solutions was examined using different organic solvents such as chloroform, dichloromethane, carbon tetrachloride, benzene, and toluene. Chloroform was found to be the most efficient organic solvent for this purpose. Double extraction with total volume 10 mL, yielding maximum and stable absorbance intensity for at least 24 h for studied drug and considerably lower extraction ability for the reagent blank and the shortest time to reach the equilibrium between both phases.

Phase ratio: Equimolar solutions was employed: a 1.0×10^{-3} M standard solution of drug base and 1.0×10^{-3} M solution of BCG, CR, and MO, respectively, were used. A series of solutions was prepared in which the total volume of drug and reagent was kept at 10 mL for BCG, CR, and MO, respectively. The absorbance was measured at the optimum wavelength. The molar ratio of the reagents (drug: dye) in the ion-pair complexes was determined by the method continuous variations (Job's method) (Figure 4). The ratio of aqueous to organic phase was ineffective and the ratio 1:1 was chosen for extraction of the

colored species. It was also noticed that the order of addition of the reagents had neither an effect on the absorbance nor the color of the complexes.

Effect of shaking and standing times (reaction time): To determine the most efficient ion-pair complex formation, shaking time of 1-5 min was studied. A constant absorbance was achieved over the examined shaking time range used and hence, 2.0 min. was chosen as an optimum shaking time throughout the experiments. Besides, the stability of the ion-pair complexes formed between the SUL and examined dyes also indicates that although the ion-pair complexes were formed instantaneously, constant absorbance readings were obtained after not less than 5 min of standing at room temperature ($25 \pm 2^\circ\text{C}$).

Composition of ion-pair complexes: The composition of ion-pair complexes was studied by Job's method of continuous variations [37] which is based on the variation of both the drug and the reagent of equal molar concentrations, keeping the total volume of the drug and the dye constant. Plots of the absorbance versus molar concentration of SUL reaches a maximum value at a mole fraction of 0.5 for each of the investigated dyes (Figure 4), which indicated that 1:1 SUL-Dye ion-pairs ($\text{SULH}^+\text{.D}^-$) were formed through the electrostatic attraction between the positive protonated drug (SULH^+) and anion dye (D^-) species.

Conditional stability constants (K_f) of the ion-pair complexes: The stability of the ion-pair complexes was evaluated. The formations of the ion-pairs were rapid and the yellow color extracts were stable for at least 24 h at room temperature without any change of either the color intensity or the maximum absorbance. The conditional stability constant (K_f) of an ion-pair complex can be calculated from the continuous variation data using the following equation:

$$K_f = (A/A_m) / [1 - A/A_m]^{n+1} C^n$$

where A and A_m are the observed maximum absorbance and the absorbance value when all the amount of drug is associated, respectively. C is the molar concentration corresponding to the maximum in absorbance and n is the stoichiometric constant with which dye ion associates with drug. Values of the obtained stability constant showed that the ion-pair complex of (SULH^+) with bromocrysol green is relatively much stable than those of methyl orange and congo-red species (Table 1).

Chromatographic studies

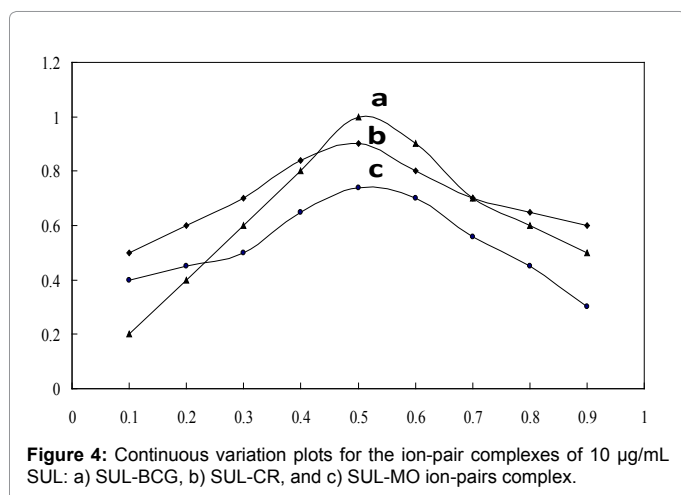


Figure 4: Continuous variation plots for the ion-pair complexes of 10 µg/mL SUL: a) SUL-BCG, b) SUL-CR, and c) SUL-MO ion-pairs complex.

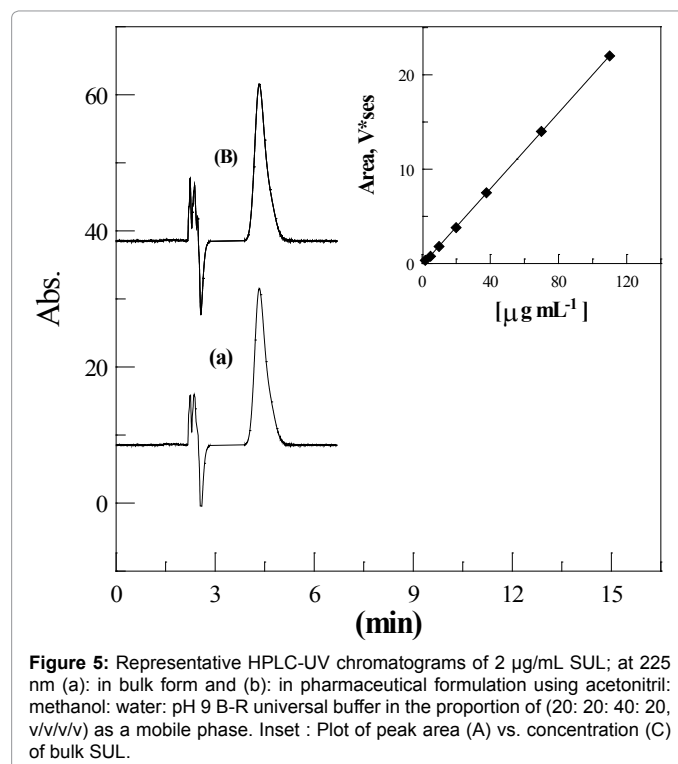


Figure 5: Representative HPLC-UV chromatograms of 2 µg/mL SUL; at 225 nm (a): in bulk form and (b): in pharmaceutical formulation using acetonitril: methanol: water: pH 9 B-R universal buffer in the proportion of (20: 20: 40: 20, v/v/v/v) as a mobile phase. Inset : Plot of peak area (A) vs. concentration (C) of bulk SUL.

The quantification of SUL was performed using a reversed phase column (Prontosil C_{18} , 250×4.0 mm, 5 µm). A number of variations such as detection wavelength, and nature, proportion and flow rate of the mobile phase were tested to achieve a suitable retention time and a symmetrical peak with a good resolution. The maximum absorption and good chromatographic response of SUL were found at 225 nm which was chosen for the rest of analysis. Several mobile phases of binary or ternary eluents with different buffers of various pH values were examined. However, a mobile phase consisting of acetonitril: methanol : water : pH 9 B-R universal buffer (20: 20: 40: 20, v/v/v/v) was found to be optimum with respect to peak shape, retention time, sensitivity and it was chosen in the rest of this study. Flow rates between 0.5 and 1.5 mL/min were studied and a flow rate of 0.6 mL/min was chosen since a signal-to-noise ratio with reasonable retention time ($t_r = 4.77$ min) were obtained (Figure 5a).

Methods validation

Linearity, limit of detection and limit of quantitation: The Beer-Lambert law limits, molar absorptivity, Sandell's sensitivity, regression equations and correlation coefficients obtained by linear square treatment of the spectrophotometric results are given in Table 1. The high molar absorptivities (2.10×10^4 - $4.10 \times 10^4 \text{ Lmol}^{-1}\text{cm}^{-1}$) of the formed colored ion-pair complexes indicated high sensitivity of the described ion-pairs extractive spectrophotometric methods for quantitation of SUL. Limits of detection (LOD) and quantitation (LOQ) of SUL were estimated from the calibration graphs using the expression $k \text{ S.D.}/b$ [38], where $k=3$ for LOD and 10 for LOQ, S.D. is the standard deviation of the blank (or the intercept of the calibration curve) and b is the slope of the calibration graph. Limits of detections of 0.044, 0.095, and 0.064 µg/mL bulk SUL were achieved by means of the three described BCG, CR, and MO methods, respectively. The results reported in Table 1, indicated the reliability of the described methods for assay of bulk SUL.

For chromatographic analysis, three calibration curves with seven

concentration points were constructed for bulk SUL. The method was proven to be linear over SUL concentration range of 0.034 to 110 µg/mL with a mean correlation coefficient of 0.9996. Figure 5a shows representative chromatograms of 2 µg/mL bulk SUL in solution. A linear calibration graph $A(V^*s)=0.22 \pm 1 \times 10^{-4}C(\mu\text{g/mL})+0.030 \pm 4 \times 10^{-4}$, where A and C are the mean peak area and concentration, respectively, was obtained over the working concentration range (Figure 5). LOD and LOQ of 0.00555 and 0.0185 µg/mL SUL, respectively, were achieved by means of the described chromatographic method.

Precision and accuracy: The precision and accuracy of the described HPLC method were examined through intra-day, inter-day, and inter-laboratory assays [39]. Accuracy; the closeness of the measured value to the true value, was expressed as percent error (relative error) (RE%), while precision; the degree of agreement among individual test results, was expressed as percentage relative standard deviation (RSD%). The mean percentage recoveries (% R), relative error (RE%), and relative standard deviations (RSD%), Table 2, indicated the high precision and accuracy of the described methods for assay of SUL.

Robustness: In regard to assay robustness [39] of the described spectrophotometric methods, influences of small variation of some of the most important procedural conditions on the recovery and the relative standard deviation of 10 µg/mL bulk SUL were studied. This included the influence of pH (± 0.2), mL reagent added (± 0.2 mL) and reaction time (5-7 min) on the ion-pair formations of SUL with BCG, CR, and MO, respectively. The obtained mean percentage recoveries and relative standard deviations (98.88 ± 0.94 to 98.45 ± 1.88) were insignificantly affected within the studied range of variation of the procedural conditions, and consequently the described spectrophotometric methods were reliable for assay of bulk SUL and they could be considered robust.

Also, the robustness of measurements by means of the described chromatographic method was evaluated by intentional minor modifications in the composition of the constituents of mobile phase ($\pm 2\%$) and rate of its flow (± 0.02). Practically, insignificant effect was observed in peak area or retention time confirming the robustness of analysis by the described chromatographic method.

Ruggedness: The results obtained by described spectrophotometric methods were found reproducible using UV-Visible spectrophotometer (Jasco, Hachioji-cho, Tokyo, Japan) Lab. (1) and Shimadzu UV-Visible spectrophotometers Model 160A (Kyto, Japan), Lab. (2) (Inter-laboratory precision) under the same operational conditions since

Procedure	Taken (µg/mL)	Within day-(n=3)			Between days-(n=3)		
		Recovery %	RSD %	RE %	Recovery %	RSD %	RE %
Spectrophotometry							
MO	8.0	100.44	0.11	0.86	98.00	1.054	-2.00
	10.0	99.56	0.09	-0.45	97.82	1.142	-2.18
	12.0	99.70	0.10	-0.91	97.95	0.98	-2.05
CR	8.0	99.57	0.07	0.51	99.23	1.18	-0.75
	10.0	99.90	0.08	0.29	98.41	0.98	-1.59
	12.0	100.54	0.12	0.24	98.72	0.94	-1.28
BCG	8.0	100.46	0.09	0.74	98.08	1.02	-1.92
	10.0	99.80	0.11	-0.60	97.65	1.12	-2.35
	12.0	99.58	0.08	0.48	98.12	0.85	-1.88
Chromatography	5.0	99.40	0.52	-0.60	98.54	0.84	-1.26
	10.0	99.83	0.43	-0.17	99.26	1.07	-0.74
	20.0	98.86	0.63	-1.14	98.22	0.55	-1.78

Table 2: Analytical accuracy and precision of determination of bulk SUL by the described spectrophotometric and chromatographic methods (n=6).

Method	Taken µg/mL	Added µg/mL	Found µg/mL	Recovery % \pm R.S.D.	F-value t-test
Spectrophotometric					
BCG	3.0		2.97	99.00 \pm 0.90 ^a	1.65 2.32
		3.0	5.95	99.33 \pm 0.72 ^b	1.06 1.93
		6.0	8.95	99.66 \pm 0.88 ^b	1.58 1.14
		9.0	12.02	100.55 \pm 0.83 ^b	0.98 0.40
		12.0	15.00	100.25 \pm 1.37 ^b	3.83 0.05
CR	3.5		3.52	100.57 \pm 0.58 ^a	1.46 0.59
		3.5	7.06	101.14 \pm 1.25 ^b	3.19 1.17
		7.0	10.55	100.43 \pm 0.66 ^b	1.12 0.27
		10.5	13.98	99.62 \pm 1.49 ^b	4.53 0.83
		14.0	17.50	99.86 \pm 0.88 ^b	1.58 0.55
MO	4.0		3.96	99.50 \pm 0.74 ^a	1.12 1.57
		4.0	7.93	99.25 \pm 0.68 ^b	1.06 2.15
		8.0	11.92	99.50 \pm 0.91 ^b	0.59 1.41
		12.0	16.01	100.42 \pm 0.43 ^b	2.78 0.29
		16.0	20.10	100.88 \pm 0.72 ^b	1.06 0.50
Chromatography					
	3.0		2.99	99.67 \pm 0.87 ^a	1.54 1.80
		3.0	5.98	99.66 \pm 0.89 ^b	1.62 1.30
		6.0	8.95	99.33 \pm 0.75 ^b	0.49 0.51
		9.0	12.02	100.33 \pm 0.92 ^b	1.73 0.03
		12.0	15.03	100.33 \pm 1.96 ^b	0.13 0.03
Reported				100.30 \pm 0.70	

(a) Using calibration curve method & (b) using standard addition method
Theoretical *F-value*=6.6 and *t-test*=2.45 at 95% confidence limit for $n_1=4$ and $n_2=4$

Table 3: Determination of SUL in dogmatil fort® tablets (200 mg/tablet) by the described spectrophotometric, and chromatographic methods compared to its determination by a reported membrane selective electrode methods [22].

there were insignificant differences between the recoveries and relative standard deviations (98.69 ± 0.19 to 98.05 ± 0.27).

Interference studies

The interference from common excipients (e.g talc, glucose, starch, sulfate, dextrose, acetate, phosphate, and magnesium stearate) usually present in formulations was examined [39] by means of the described spectrophotometric and chromatographic methods. The mean percentage recoveries (%R) and relative standard deviations (RSD%) obtained by the three described spectrophotometric methods in the absence (98.74 ± 2.66 to $99.06 \pm 2.33\%$) and in the presence of excipients (98.16 ± 2.02 to $98.75 \pm 2.25\%$) indicated insignificant interference from excipients. Since, the formation of the ion-pair complex with the anionic dye requires the presence of a basic functional group in the analyte molecule; therefore no possible interference is likely to occur from co-formulated drugs lacking a basic center.

On the other side, insignificant interference from excipients was found by the described chromatographic method, since recovery of SUL in the absence and in the presence of excipients were 98.48 ± 2.14 and 97.87 ± 2.25 , respectively. The results suggested the specificity of the described spectrophotometric and chromatographic methods for assay of SUL. It can be seen that the proposed methods show superior selectivity behavior and exhibit a better linear response range than many of these previously suggested methods (Table 5).

Analysis of dogmatil fort® tablets

The described specrophotometric and chromatographic methods have been successfully applied to the determination of SUL in dogmatil fort® tablets using the calibration curve method (Table 3). Figure 5b shows representative chromatograms of 2 µg/mL SUL in solution of

Method	Concentration of sulpiride (µg/mL)	Recovery (µg/mL)	Recovery*, % ± R.S.D
Spectrophotometric			
BCG	14	13.92	99.43 ± 0.87
	17	16.98	99.88 ± 0.76
	20	19.96	99.80 ± 0.92
CR	14	13.88	99.14 ± 0.80
	17	16.90	99.41 ± 0.86
	20	19.94	99.70 ± 0.85
MO	14	13.90	99.28 ± 0.82
	17	16.91	99.47 ± 0.66
	20	19.92	99.60 ± 0.86
Chromatography			
BCG	14	13.94	99.57 ± 0.79
CR	17	16.93	99.59 ± 0.71
MO	20	19.97	99.85 ± 0.82

*Average of 5 measurements

Table 4: Recovery of sulpiride added to human plasma after extraction using chloroform.

Methods	Concentration range µg/mL	Literatures
HPLC and HPTLC	5-60	[10]
Synchronous Fluorescence Spectroscopy	0.05-1.0	[41]
Electrochemiluminescence	0.034-34.14	[24]
HPLC Spectrophotometry	0.034-110 2-16	The present work

Table 5: Comparison of linear range for SUL with different classical methods and the present methods.

dogmatil fort® tablets which is matching well with that of 2 µg/mL bulk SUL sample (Figure 5a). For further confirmation, the standard addition method was applied to test the reliability and recovery of the described methods. This was carried out by analysis known concentrations of SUL added to a previously analyzed solution of dogmatil fort® tablets. The results obtained by the described spectrophotometric and HPLC methods were statistically compared with those obtained using a reported membrane selective electrode method [22] (Table 3). The calculated student's t-values and F-values did not exceed the theoretical ones at 95% confidence level [40] indicating no significant difference between the described spectrophotometric and chromatographic and the reported method [22] regarding accuracy, precision and reproducibility.

Analysis of sulpiride in human plasma

The ability of the proposed method to determine SUL in plasma has been appraised through spiking plasma samples with the drug at different concentration levels. It was found that SUL could be estimated with good recoveries (Table 4) at the levels of 14-20 µg/mL plasma, thus indicating that there is no interference from endogenous constituents [41].

Conclusions

Three validated extractive spectrophotometric methods were described for determination of SUL in pharmaceutical formulations and plasma as ion pair colored complexes with BCG, CR and MO. Each of the ion-pair complexes was quantitatively extracted in chloroform in one step and was stable for at least 24 h. Besides a simple reversed phase HPLC with UV detection method was also developed. No significant interference from the common excipients was found by

the described spectrophotometric and chromatographic methods. The high recovery and low relative standard deviation reflect the high accuracy and precision of the described spectrophotometric and chromatographic methods for assay of SUL. Moreover, the methods are simple, precise, applicable to a wide range of concentration, besides being less time consuming and depending on simple and available reagents thus offering economic and acceptable methods for the routine determination of SUL in pharmaceutical formulations and plasma. The comparative study of the molar absorptivity indicated good sensitivity of the proposed method which follow the order of BCG>MO>CR.

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